MCLAB
Molecular Cloning Laboratories

User Manual

Version 1.0
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Product name: BL21(DE3) Competent E. Coli

Cat #: BS-100, BS-196

Description:
High yields, high efficiency, no heat shock necessary.

Recommended Storage Conditions:
This product should be stored at -80°C. Thaw on ice only before use. Do not re-freeze.

Transformation Steps
1. Thaw one vial of cells on ice per transformation.

2. Add 5–10 ng of DNA, in a volume of 1–5 µL to the cells and mix by tapping gently. Do not mix cells by pipetting.

3. Incubate the vial(s) on ice for 30 minutes.

4. Heat shock the cells by incubating the vial(s) for exactly 30 seconds in the 42°C water bath. Do not mix or shake.

5. Remove the vial(s) from the 42°C bath and quickly place on ice.

6. Add 250 µL of pre-warmed SOC medium to the vial(s). (SOC is a rich medium; use proper sterile technique to avoid contamination.)

7. Secure the vial(s) in a microcentrifuge rack with tape. Place the rack in a shaking incubator, and shake the vial(s) at 37°C for 1 hour at 225 rpm.

8. Plate two different volumes of the transformation reaction onto LB plates containing the appropriate antibiotic for plasmid selection. Include 34 µg/mL chloramphenicol if using BL21(DE3) pLysS or BL21(DE3)pLysE cells. Select two volumes ranging from 20–200 µL to ensure well-spaced colonies on at least one plate. The remaining transformation reaction may be stored at 4°C and plated out the next day, if needed.

9. Invert the plates and incubate at 37°C overnight.

10. Select transformants from the plates and culture as described on page 8.
Note: Clones may exhibit differences in expression of heterologous genes. We recommend choosing 3–4

transformants when characterizing clones for protein expression.

Expression Steps

1. Pick 3–4 transformants for overnight culture in 5 mL LB medium containing antibiotic to select for your expression plasmid. If using BL21(DE3)pLysS or BL21(DE3)pLysE, add 34 µg/mL chloramphenicol to select for pLysS or pLysE. Grow overnight at 37°C with shaking to saturation (OD600 ≥2).

2. Use the overnight cultures to inoculate fresh LB medium containing antibiotic to an OD600 of 0.05–0.1 (~1:50 dilution of the overnight culture). This dilution allows the cells to quickly return to logarithmic growth and reach the appropriate cell density. Use a volume appropriate for taking time points, if desired.
Note: If you are using BL21(DE3)pLysS or BL21(DE3)pLysE, you may choose not to include chloramphenicol in these cultures. Generally, the cells will not lose the pLysS or pLysE plasmid during the limited number of cell doublings that occur in the growth and induction stages.

3. Use the remainder of each overnight culture to create glycerol stocks. Once you have identified the clone that
best expresses your protein, you can use the glycerol stock to perform additional expression experiments.

4. Grow the cultures until they reach mid-log phase (OD600 ~0.4; 2 to 3 hours).

5. Induce the cultures by adding IPTG to a final concentration of 0.5 mM and culture for an additional 2–3 hours. You may also take time points to analyze for optimal expression of your protein.

6. Analyze clones by western blot or enzymatic assay to determine which clone best expresses your protein of interest. Use the glycerol stock created from this clone for expression experiments. If you find that expression levels in subsequent inductions decrease, or you find that you lose your plasmid, your protein may be toxic to E. coli.

Expression Guidelines

1. Use BL21(DE3)pLysS or BL21(DE3) pLysE strains for expression experiments. Both strains produce T7 lysozyme to inhibit the action of T7 RNA polymerase and reduce basal level expression of the gene of interest. Choose BL21(DE3)pLysE when the tightest regulation of expression is required.

2. Propagate and maintain your expression plasmid in a strain that does not contain T7 RNA polymerase (i.e. DH5α, JM109, etc.).

3. Perform a fresh transformation of BL21(DE3)pLysS or BL21(DE3)pLysE cells before each induction experiment.

4. Minimize the amount of time that the cells bearing the gene of interest are cultured before IPTG induction.

5. Following transformation of BL21(DE3)pLysS or BL21(DE3)pLysE cells, grow cells in SOC medium for 1 hour and go directly to protein expression. Do not plate the transformation mixture to select for individual clones.
See next page for details.